abcam

Product datasheet

Human TPBG knockout MCF7 cell lysate ab269661

4 Images

Overview

Product name	Human TPBG knockout MCF7 cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	MCF7
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 1 bp insertion; Frameshift: 97%
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS), Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
Notes	**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared. **User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage. Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. **See here for more information on knockout cell lysates.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses. This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.
Tested applications	**Suitable for:** WB

Properties

Properties

Storage instructions	Store at -80°C. Please refer to protocols.

Components	1 kit
ab280536 - Human TPBG knockout MCF7 cell lysate	1 x 100µg
ab269599 - Human wild-type MCF7 cell lysate	1 x 100µg

Cell type	epithelial
Disease	Adenocarcinoma
Gender	Female

Target

Tissue specificity	Expressed by all types of trophoblasts as early as 9 weeks of development. Specific for trophoblastic cells except for amniotic epithelium. In adult tissues, the expression is limited to a few epithelial cell types but is found on a variety of carcinoma.
Sequence similarities	Contains 6 LRR (leucine-rich) repeats. Contains 1 LRRCT domain. Contains 1 LRRNT domain.
Cellular localization	Membrane.
Form	5T4 is a 72 kDa transmembrane glycoprotein that exhibits restricted expression in human and mouse adult tissues, is upregulated on many carcinomas and tumour expression correlates with poorer clinical outcome in ovarian, gastric and colorectal cancers. mES cells lack cell surface expression of the 5T4 antigen, and both protein and mRNA are rapidly upregulated following induction of differentiation. This proteins expression correlates with downregulation of OCT-4 and Tra-1–60 (Ward et al., 2006).

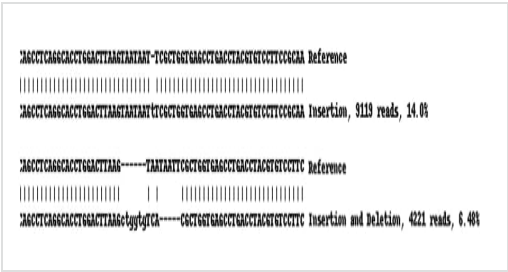
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab269661 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

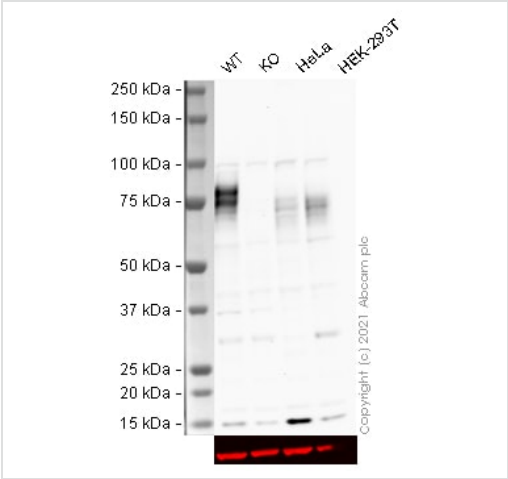
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 46 kDa.

Images



Next Generation Sequencing - Human TPBG knockout MCF7 cell lysate (ab269661)

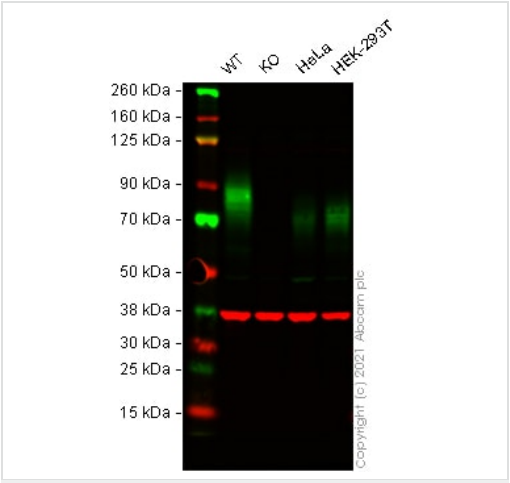
Knockout achieved by CRISPR/Cas9; X = 1 bp insertion; Frameshift: 97%



Western blot - Human PCBG knockout MCF7 cell lysate

Lane 1: Wild-type MCF7 (Human breast adenocarcinoma cell line) whole cell lysate, 20 ug
Lane 2: TPBG knockout MCF7 (Human breast adenocarcinoma cell line) whole cell lysate, 20 ug
Lane 3: HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate, 20 ug
Lane 4: HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate, 20 ug

ab199547 was shown to react with 5T4 (HRP) in wild-type MCF7 cells in western blot. Loss of signal was observed when TPBG knockout cell line **ab269499** (knockout cell lysate ab269661) was used. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab199547** overnight at 4 °C at a 1 in 5000 dilution Blots were developed with Optiblot ECL reagent (**ab133456**) and imaged.

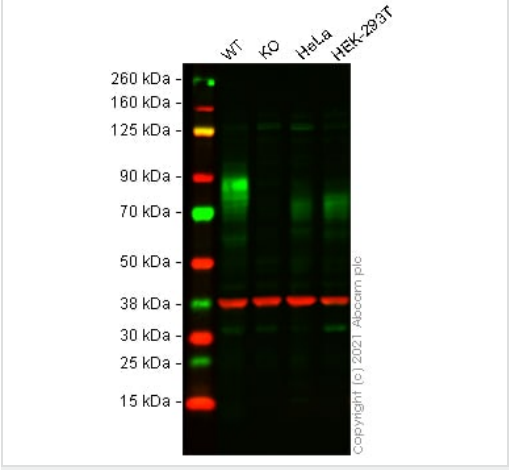


Western blot - Human TPBG knockout MCF7 cell lysate

Lane 1: Wild-type MCF7 (Human breast adenocarcinoma cell line) whole cell lysate, 20 ug
Lane 2: TPBG knockout MCF7 (Human breast adenocarcinoma cell line) whole cell lysate, 20 ug
Lane 3: HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate, 20 ug
Lane 4: HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate, 20 ug

Lanes 1 - 4: Merged signal (red and green). Green - **ab134162** observed at 85 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab134162 was shown to react with 5T4 in wild-type MCF7 cells in Western blot with loss of signal observed in TPBG knockout cell line **ab269499** (knockout cell lysate ab269661). Wild-type MCF7 and TPBG knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in before incubation with **ab134162** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Human TPBG knockout MCF7 cell lysate

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Lane 3: HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate, 20 ug
Lane 4: HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate, 20 ug

Lanes 1 - 4: Merged signal (red and green). Green - **ab129058** observed at 85 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab129058 was shown to react with 5T4 in wild-type MCF7 cells in Western blot with loss of signal observed in TPBG knockout cell line **ab269499** (knockout cell lysate ab269661). Wild-type MCF7 and TPBG knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in before incubation with **ab129058** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L

(IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

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